

Alpha₁-antitrypsin deficiency with M-like phenotype¹

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SUMMARY A patient with a low serum concentration of α_1 -antitrypsin (0.1 g/l) but with an M-like phenotype is described. Her parents and 2 sibs have a Pi^M phenotype, but all except the father have approximately half-normal levels of α_1 -antitrypsin: The M-like variant apparently cannot be distinguished from M- α_1 -antitrypsin, when it occurs with M in heterozygotes. The proposita has severe airways obstruction and emphysema, and her father has moderate chronic obstructive pulmonary disease. The mother and 2 sibs are healthy.

Since the first descriptions of α_1 -antitrypsin deficiency associated with obstructive lung disease and emphysema by Eriksson (1964, 1965) many cases of this syndrome have been reported. Most of these patients are homozygous for the allele Pi^Z. A few other types, however, have been described: Talamo *et al.* (1973) reported a patient with no detectable α_1 -antitrypsin in his serum. Cox (1975), Lieberman *et al.* (1976) and Martin *et al.* (1975) have found patients with low concentrations of α_1 -antitrypsin which was of the same or similar electrophoretic mobility as the M protein. In this report, we describe a patient with a low serum level of α_1 -antitrypsin whose phenotype however, was different from Z but similar to M.

Subjects and methods

FAMILY

The proposita is a 38-year-old woman. At the age of 12 years she had pneumonia. She never smoked and had no chronic bronchitis. At the age of 20 years, she noticed dyspnoea on exertion; a chest x-ray film at that time was interpreted as showing emphysema of the right lung, and an electrocardiogram showed signs of right ventricular hypertrophy. At the present examination, she was dyspnoeic at rest. A chest x-ray film showed a decrease in vascular markings of the right lung and at the left base. A lung scintigram using ¹²⁵I-labelled macroaggregated albumin showed defects in perfusion over the whole

right lung and the left base. Pulmonary function tests indicated a greatly reduced vital capacity (VC) of 1.05 litres (31% of the predicted normal value), and the forced expiratory volume in 1 second (FEV₁) was 0.35 litre, which was only 33% of her VC and 10% of the predicted normal VC. The pressure in the pulmonary artery was increased to 66/45 torr, the P_aO₂ was 35 torr, and the P_aCO₂ was 57 torr, when the patient was at rest and breathing room air. These data indicate severe airway obstruction and are compatible with advanced emphysema.

The 69-year-old father of the proposita had somewhat decreased vascular markings at both bases on a chest radiograph and a moderate perfusion defect in the same areas on a perfusion scan. His VC was 3.03 litres (81% of predicted normal) and his FEV₁ was 1.89 litres, which was 62% of his VC and 50% of the predicted normal VC; these values indicated mild airway obstruction. He has smoked cigarettes since he was in his teens and currently smokes one packet a day.

A chest x-ray film pulmonary function tests, and scintigraphy also were done on the mother, 1 sister, and 1 brother, all smokers. None had pulmonary emphysema or airway obstruction.

TECHNIQUES

Trypsin inhibiting activity was measured according to a previously published method (Briscoe *et al.* (1966)). The proportion of active trypsin in the preparation used (Boehringer Mannheim, 15330 ETAB) was determined by active site titration (Chase and Shaw, 1967). The trypsin inhibiting activity is expressed as the amount of active trypsin inhibited by 1 ml of serum. The mean value for serum samples with

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an M phenotype is 0.64 mg of trypsin inhibited by 1 ml of serum.

Radial immunodiffusion was done according to the method of Mancini *et al.* (1965), as described previously (Kueppers, 1967).

Immunoelectrophoresis was performed according to the micromethod described by Hirschfeld (1959).

Starch gel electrophoresis with subsequent antigen-antibody-crossed electrophoresis was done as described (Fagerhol, 1972; Laurell, 1965).

Isoelectric focusing pH 3.5 to 5.0 was done in the Multiphor (LKB 2117) according to the accompanying application note (Karlsson *et al.*, 1973). Double diffusion was done according to the method of Ouchterlony (1958). For Gm typing the procedure of Grubb (1970) was used.

Table 1 *Trypsin inhibiting activity, alpha₁-antitrypsin values and Gm types*

	Age (y)	α_1 -Antitrypsin level (g/l)	Trypsin inhibited†	Probably Gm genotype
Proposita	38	0.11	0.03	ag/fb
Father	69	1.90	0.54	fb/fb
Mother	61	0.76	0.16	ag/fb
Sister	23	1.49	0.46	fb/fb
Brother	21	1.03	0.20	ag/fb

*Normal range for M phenotype, 1.80 to 2.50 g/l Fagerhols standard serum pool contains 2.23g/l by radial immunodiffusion when measured as described (Kueppers, 1967).

†Milligrammes of trypsin inhibited by 1 ml of serum; normal range for M phenotype; 0.5 to 0.72 mg.

Results

The quantitative results of the trypsin inhibiting activities and the α_1 -antitrypsin concentration as determined by radial immunodiffusion and the Gm types are given in the Table.

Acid-starch gel electrophoresis of the proposita's serum did not reveal any banding pattern in the position typical for M- α_1 -antitrypsin. Sera of all other first-degree relatives showed a pattern typical for the common M phenotype. Antigen-antibody-crossed electrophoresis of the thrice-concentrated serum of the proposita after starch gel electrophoresis showed precipitating material in the approximate position of M- α_1 -antitrypsin, or perhaps a little more anodally; the shape of the precipitate was also similar to that of M (Fig. 1). All first-degree relatives showed the typical M pattern on antigen-antibody-crossed electrophoresis.

Isoelectric focusing pH 3.5 to 5.0 confirmed the observations of the starch electrophoretograms. The proposita's serum showed diffuse bands in the M position. All other relatives had regular M bands of α_1 -antitrypsin that differed in concentration only.

Immunoelectrophoresis clearly showed that the α_1 -antitrypsin of the proposita migrated slightly faster toward the anode than did the M protein and still faster than did the Z variant (Fig. 2).

No immunological difference between the new variant and M- α_1 -antitrypsin could be shown by Ouchterlony double diffusion using antisera from several rabbits.

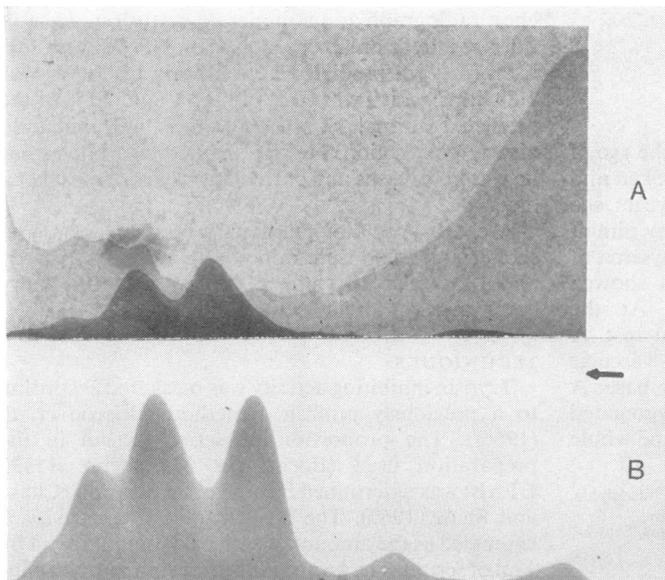


Fig. 1 *Antigen-antibody-crossed electrophoresis of proposita's serum 3x concentrated (A) and her father's serum (B), which shows a regular PiM phenotype. Anode is at the left.*

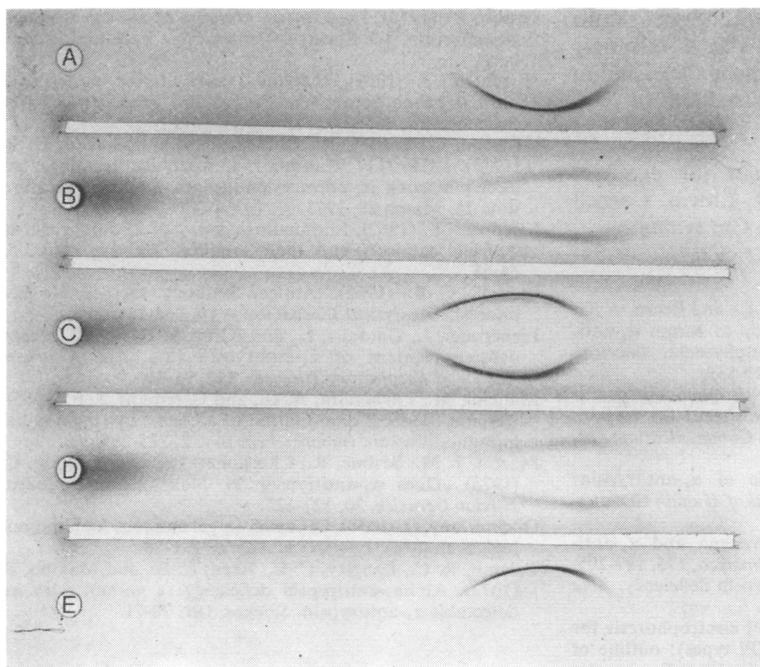


Fig. 2 Immunoelectrophoresis of the proposita's serum (B, D), a 1/5 diluted serum with a Pi^M phenotype (A, E) and a serum with Pi^Z phenotype (C). Anti-serum specific against α_1 -antitrypsin in all troughs. Anode is at the right.

Discussion

Our data indicate that this type of α_1 -antitrypsin is clearly different from the common variants described so far. In particular, it is distinct from the M protein on immunoelectrophoresis (Fig. 2) and from Z protein on isoelectric focusing and immunoelectrophoresis, though there is some overlap with the M pattern on antigen-antibody-crossed electrophoresis (Fig. 1). This overlap accounts for the fact that putative heterozygotes for Pi^M and this variant are indistinguishable from Pi^M homozygotes and heterozygotes Pi^M/Pi^- . Therefore, we cannot be certain about the genotypes in this family: the proposita may be homozygous for the new variant or may be heterozygous for the variant allele and Pi^- . The presence of a Pi^- allele also cannot be excluded in other family members. Though the proposita's father has an α_1 -antitrypsin concentration in the normal range, we should assume (provided the proposita does not represent a new mutation) that he is heterozygous for an allele determining a low α_1 -antitrypsin level and that this level at the time of this study was only temporarily raised because of his disease.

It is tempting to speculate that the mother and father are heterozygous for different deficiency alleles determining differently low levels and that in the mother the allele determining the lower α_1 -antitrypsin concentration is in coupling with Gm ag:

the proposita as well as her brother may have received from their mother the chromosome containing the gene for Gm ag and the deficiency allele. Her sister could have received Gm f b and Pi^M from the mother and Gm f b with a deficiency allele from her father. This assumption could explain the higher α_1 -antitrypsin concentration in the sister as compared with that in her brother and mother. The loose linkage of the Pi and Gm loci is well known (Gedde-Dahl *et al.*, 1972).

Recently Cox (1975) and Lieberman *et al.* (1976) have reported a family with an α_1 -antitrypsin variant with a low serum concentration that had a mobility similar or identical to that of M. Those variants may be identical to the phenotype described here. If comparison of serum samples shows that the 3 variants are identical, a common name should be used.

The proposita had clear evidence of airways obstruction and emphysema. Her father, now 69 years old, had only moderate obstruction. His perfusion lung scan showed defects at both bases, compatible with emphysema. He has smoked cigarettes since his teen-age years and at present smokes 1 packet a day. This is perhaps an example of a heterozygote in whom emphysema developed as a consequence of long-standing bronchial and alveolar irritation by tobacco smoke.

The α_1 -antitrypsin type found in this family shows the heterogeneity of α_1 -antitrypsin deficiency.

It is important to be aware of the occurrence of this allele or similar ones in families with a seemingly anomalous inheritance in the Pi system alleles and in some patients with airways obstruction or emphysema.

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